

Supplementary Materials for
**A novel tRF-Gly is associated with obesity development through epigenetic
regulation of lipid metabolism**

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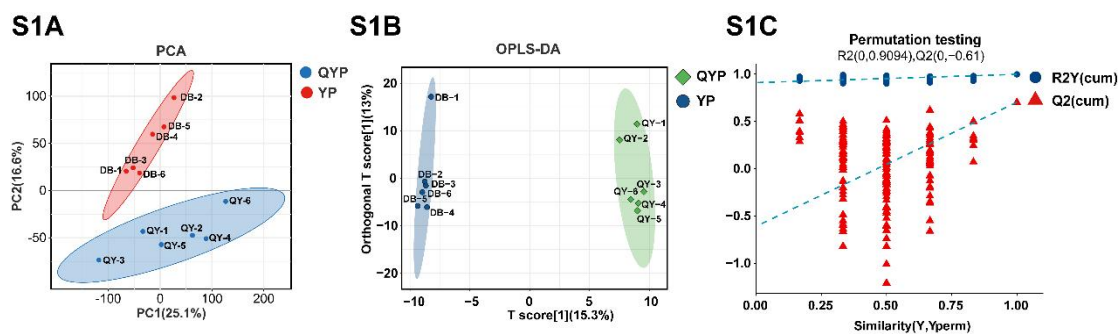


Fig. S1. LC / MS non-targeted metabolomic analysis of visceral fat in YP and QYP pigs. (A) PCA analysis of VAT adipose lipid composition in YP and QYP pigs (n=6). (B) OPLS-DA analysis of VAT adipose lipid composition in YP and QYP pigs (n=6). (C) Permutation testing confirmed the high reliability of the metabolome sequencing results (n=6).

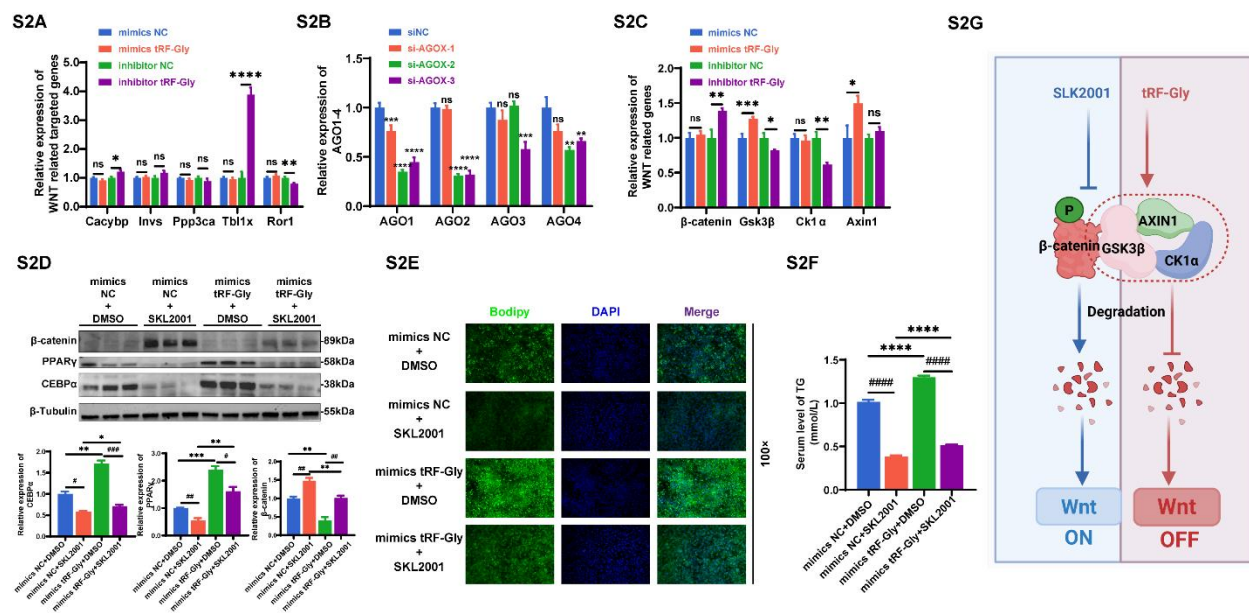


Fig. S2. tRF-Gly modulated the Wnt/β-catenin signaling pathway. (A) The expression level of the 10 predicted target genes associated with the Wnt/β-catenin signaling pathway in the differentiation stage of 3T3-L1 adipocyte after transfection of the tRF-Gly mimic and inhibitor. (B) The knockdown efficiency of three independent siRNA candidates targeting AGO1, AGO2, AGO3, and AGO4 was evaluated by qRT-PCR. (C) The expression level of genes associated with the Wnt/β-catenin signaling pathway in the differentiation stage of 3T3-L1 adipocyte after transfection of the tRF-Gly mimic and inhibitor. (D) Representative immunoblotting and quantification of β-catenin, PPARγ, and CEBPα protein levels in 3T3-L1 adipocytes following tRF-Gly overexpression under SKL2001 treatment. (E) Representative Bodipy staining of lipid droplet accumulation in 3T3-L1 adipocytes following tRF-Gly overexpression under SKL2001 treatment (100x, 100 μm). (F) Triglyceride biochemical assay in 3T3-L1 adipocytes following tRF-Gly overexpression under SKL2001 treatment. (G) Schematic diagram of the regulatory effects of tRF-Gly and SKL2001 on the Wnt/β-catenin signaling pathway. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$. All results above are representative of three independent experiments and presented as the means $\pm$ SEM. Abbreviations: AGOX, AGO1/2/3/4; β-catenin, Catenin Beta 1; GSK3β, Glycogen Synthase Kinase 3 Beta; CK1α, Casein Kinase 1 Alpha 1.

Table S1. The sequences of RNA oligonucleotide

Names	Sequence 5'-3'
mimics NC	Sense: UUCUCCGAACGUGUCACGUTT
	Antisense: ACGUGACACGUUCGGAGAATT
tRF-Gly mimics	Sense: GCAUUGGUGGUUCAGUGGUAGAAUUCUCGCC
	Antisense: CGAGAAUUCUACCACUGAACCACCAAUGCUU
inhibitor NC	Sense: CAGUACUUUUGUGUAGUACAA
tRF-Gly inhibitor	Sense: GGCGAGAAUUCUACCACUGAACCACCAAUGC

Table S2. The sequences of Small interfering RNA (siRNA)

Names	Sequence 5'-3'
si-NC	Sense: UUCUCCGAACGUGUCACGU
	Antisense: ACGUGACACGUUCGGAGAA
si-AGO1-1	Sense: GCUACAACCUGGAUCCCUA
	Antisense: ACGUGACACGUUCGGAGAATT
si-AGO1-2	Sense: GGUUCACCAGGAUACUCUA
	Antisense: UAGAGUAUCCUGGUGAACC
si-AGO1-3	sense: GCUCAACAUUGAUGUCUCA
	Antisense: TUGAGACAUCAAUGUUGAGC
si-AGO2-1	Sense: GCACAACCGUGGACACGAA
	Antisense: UUCGUGUCCACGGUUGUGC
si-AGO2-2	Sense: GGUACCACCUGGUGGAUAA
	Antisense: UUAUCCACCAGGUGGUACC
si-AGO2-3	Sense: CUAUGAAUUGGACAUCAAA
	Antisense: UUUGAUGUCCAAUUCAUAG
si-AGO3-1	Sense: CAAGAUACCUUACGCACAA
	Antisense: UUGUGCGUAAGGUAUCUUG
si-AGO3-2	Sense: GGACAAUCAGACUUCUACA
	Antisense: UGUAGAAGUCUGAUUGUCC
si-AGO3-3	Sense: CUAUGAAGUUGACAUCAAA
	Antisense: UUUGAUGUCAACUUCAUAG
si-AGO4-1	Sense: GGAGCUAAUAGCAAUUCGA
	Antisense: UCGAAUUGCUAUUAGCUCC
si-AGO4-2	Sense: GCACUUGAUGUGAUUACAA
	Antisense: UUGUAAUCACAUCAAGUGC
si-AGO4-3	Sense: CACUGACCAACUUCGUAAA
	Antisense: UUUACGAAGUUGGUCAGUG
si-RAC1	Sense: CUCAUCAGUUACACGACCAAU
	Antisense: AUUGGUCGUGUAACUGAUGAG

Table S3. The primer sequences for qRT-PCR assay

Names	Primer	Sequence 5'-3'
Pcna	Forward	TTTGAGGCACGCCTGATCC
	Reverse	GGAGACGTGAGACGAGTCCAT
Ccnd1	Forward	AGAGGCGGATGAGAACAAGCA
	Reverse	GCGGTAGCAGGAGAGGAAGTT
Bax	Forward	TGAAGACAGGGGCCTTTTTG
	Reverse	AATTCGCCGGAGACACTCG
Bcl2	Forward	GTCGCTACCGTCGTGACTTC
	Reverse	CAGACATGCACCTACCCAGC
Ccbp α	Forward	CAAGAACAGCAACGAGTACCG
	Reverse	GTCACTGGTCAACTCCAGCAC
Ppar γ	Forward	TCGCTGATGCACTGCCTATG"
	Reverse	GAGAGGTCCACAGAGCTGATT
Aacac	Forward	GATGAACCATCTCCGTTGGC
	Reverse	GACCCAATTATGAATCGGGAGTG
Adipoq	Forward	TGTTCTCTTAATCCTGCCCA
	Reverse	CCAACCTGCACAAGTTCCTT
Scd1	Forward	GTGAGACCTGATACCTAACA
	Reverse	AAGGCTCTGATTCTACAAGT
Plin2	Forward	GACCTTGTGTCCTCCGCTTAT
	Reverse	CAACCGCAATTTGTGGCTC
Ago1	Forward	CAGCAGGTGTTTCAGGCAC
	Reverse	GGACACTTATCCGGCTTGATG
Ago2	Forward	CGTCCTTCCCACTACCACG
	Reverse	CCAGAGGTATGGCTTCCTTCA
Ago3	Forward	GCCCAGCCCCTATTCATCG
	Reverse	ACTCTTCGAGGACACTTGTCT
Ago4	Forward	CCAATTCGACTGTTAGCCAATCA
	Reverse	CGAGGCCGTTTTTCTGGTTTAAT
β -catenin	Forward	ATGGAGCCGGACAGAAAAGC

	Reverse	CTTGCCACTCAGGGAAGGA
	Forward	TGGCAGCAAGGTAACCACAG
Gsk3 β	Reverse	CGGTTCTTAAATCGCTTGTCTG
	Forward	ACTGAGGTATTAGGGTGCAGC
Axin1	Reverse	GCATCTTCGGTGAAACTTGCTC
	Forward	TCCAAGGCCGAATTTATCGTC
Ck1 α	Reverse	ACTTCCTCGCCATTGGTGATG
	Forward	TAAATACCGGGTGTTTATTGGGC
Map3k1	Reverse	TTTTCTCCATAACATGGGGTCAG
	Forward	AATCGACAGCACGGTTTACTC
Map2k4	Reverse	TGAAATCCCAGTGTTGTTTCAGG
	Forward	GATGTCGCGTCCTGGTTTAAG
Map2k7	Reverse	ACTTGGGAGAAGGTGGGGAA
	Forward	TCAGTGGGTTGCATCATGGG
Jnk2	Reverse	GGATGGTGTTCTTAGCTGTTCA
	Forward	GTCCGTGCAAAGTGGTATCCT
Rac1	Reverse	GCACCGATCTCTTTCGCCAT
	Forward	AGGTCGGTGTGAACGGATTTG
Gadph	Reverse	TGTAGACCATGTAGTTGAGGTCA
	Forward	GGCTGTATTCCCCTCCATCGA
β -actin	Reverse	CCAGTTGGTAACAATGCCATGT
	Forward	GCATTGGTGGTTCAGTGGTAGAATTCTCGCC
tRF-Gly	Reverse	Uni-miR qPCR Primer, included in kit (TaKaRa)
	Forward	CTCGCTTCGGCAGCACA
U6	Reverse	AACGCTTCACGAATTTGCGT

Table S4. Northern blot probe sequences.

Gene name	Sequence (5'-3')
Biotin-U6	TGGAACGCTTCACGAATTTG
Biotin-tRF-Gly	GGCGAGAATTCTACCACTGAACCACCAATGC TGGTGCATTGGCCGGGAATCGAACCCGGGCCT
Biotin-tRNA-Gly-GCC	CCCGCGTGGCAGGCGAGAATTCTACCACTGAA CCACCAATGC